Table of Contents

Introduction

Key to the kit is our proprietary DNA binding systems that DNA exclusively and efficiently binds to our ezBind™ matrix while proteins and other impurities are removed by wash buffer. Nucleic acids are easily eluted with sterile water or Elution Buffer.Unlike other kits in the markets, no chaotropic salts are contained in the buffer of our patented plasmid purification kit. The purified DNA is guanidine/anion exchange resin residues free.

Plasmid isolated with traditional protocol normally contains high level of endotoxins (Lipopolysaccharides or LPS). For transfection of endotoxin sensitive cell lines or microinjection, the endotoxins should be removed before the applications. The EZgene™ endofree system uses a specially formulated buffer that extracts the endotoxin from the plasmid DNA. Two rounds of extraction will reduce the endotoxin level to 0.1 EU (Endotoxin) per μg of plasmid DNA. The endofree plasmid miniprep kit provides an efficient endotoxin removal step into the traditional purification procedure to produce transfection grade plasmid DNA.

This kit is designed for fast and efficient purification of plasmid DNA from 15 to 50 mL of *E. coli* culture. The mini column has a DNA binding capacity of 250 μg.

The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Important Notes

Plasmid Copy Numbers: The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 2 times. Please contact our customer service for further information and reference Table 1 for the commonly used plasmids,

Table 1 Commonly used plasmid.

Plasmid	Origin	Copy Numbers	Expected Yield (μg per 50 mL)
pSC101	pSC101	5	5
pACYC	P15A	10-12	5-10
pSuperCos	pMB1	10-20	10-20
pBR322	pMB1	15-20	10-20
pGEM®	Muted pMB1	300-400	100-150
pBluescript®	ColE1	300-500	100-200
pUC	Muted pMB1	500-700	150-250

Host Strains: The strains used for propagating plasmid have significant influence on yield. Host strains such as Top 10 and DH5a yield high-quality plasmid DNA. *endA*+ strains such as JM101, JM110, HB101, TG1 and their derivatives, normally have low plasmid yield due to either endogenous endonucleases or high carbohydrates released during lysis. We recommend transform plasmid to an *endA*- strain if the yield is not satisfactory.

Table2 *endA* strains of *E. Coli*.

EndA*- Strains of *E. Coli							
DH5α	DH1	DH21	JM106	JM109	SK2267	SRB	XLO
TOP10	DH10B	JM103	JM107	SK1590	MM294	Stbl2™	XL1-Blue
BJ5182	DH20	JM105	JM108	SK1592	Select96™	Stbl4™	XL10-Gold

EndA*+ Strains of *E. Coli							
C600	JM110	RR1	ABLE® C	CJ236	KW251	P2392	BL21(DE3)
HB101	TG1	TB1	ABLE® K	DH12S ™	LE392	PR700	BL21(DE3) pLysS
JM101	JM83	TKB1	HMS174	ES1301	M1061	Q358	BMH 71-18
All NM strains				All Y strains			

Optimal Cell Mass (OD_{600} x mL of Culture): This procedure is designed for isolating plasmid grown in standard LB medium (Luria Bertani) for 12 -16 hours to a density of OD_{600} 2.0 to 3.0. If rich mediums such as TB or 2xYT are used, make sure the cell density doesn't exceed 3.0 (OD_{600}). A high ratio of biomass over lysis buffers result in low DNA yield and purity. The midi column has an optimal biomass of 100-150. For example, if the OD_{600} is 3.0, the optimal culture volume should be 25-50 mL.

Culture Volume: Use a flask or tube 4 times bigger in voulme than the culture medium to secure optimal condition for bacteria growth. Don't exceed the maximum culture volume suggested in the protocol. Incomplete lysis due to over amount of bacterial culture results in lower yield and less purity.

Storage and Stability

Buffer A1 should be stored at 4℃ once RNase A is added. All other materials can be stored at room temperature (22-25℃). The Guaranteed shelf life is 12 months from the date of purchase.

Before Starting

Two endotoxin removal procedures are provided. Protocol A removes endotoxin during the purification of plasmid DNA and Protocol B removes endotoxin after the purification of plasmid DNA.

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each step.

Important

- RNase A: It is stable for more than half a year when stored at room temperature. Spin down the RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use. Store at 4℃.
- Buffer ER should be stored at 4℃.
- Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 50℃ to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure the availability of centrifuge, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation.
- Carry out all centrifugations at room temperature.

Materials supplied by users

- 70% ethanol and 100% ethanol.
- High speed centrifuge.
- 30 mL high speed centrifuge tubes.
- 15 mL and 50 mL conical tubes.

Kit Contents

Catalog #	**PD1420-00**	**PD1420-01**	**PD1420-02**
Preps	2	10	25
EzBind™ Columns	2	10	25
Buffer A1	6 mL	30 mL	70 mL
Buffer ER	300 µL	1.5 mL	3.5 mL
Buffer B1	6 mL	30 mL	70 mL
Buffer D1	600 µL	3 mL	7 mL
Buffer N3	3 mL	15 mL	30 mL
Buffer RET	12 mL	60 mL	135 mL
Endofree Elution Buffer	3 mL	15 mL	40 mL
RNase A (20 mg/mL)	0.6 mg (30 µL)	3 mg (150 µL)	7 mg (350 µL)
User Manual	1	1	1

Safety Information

- Buffer N3 contain acetic acid, wear gloves and protective eyewear when handling.
- Buffer N3 and RET contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

EZgene ™ EndoFree Plasmid ezFlow Midiprep Spin Protocol:

1. Inoculate **15-50 mL** LB containing appropriate antibiotic with 50 µL fresh starter culture. Inoculate at 37 ℃ for 14-16 hours with vigorous shaking.

   Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 ml LB medium containing the appropriate antibiotic and grow at 37 ℃ for 6-8 hours with vigorous shaking (~250 rpm).
   Note: Do not use more than 50 mL culture or cell mass greater than 150. The buffer volume needs to be scaled up if processing over 100 mL of culture.
   Note: Do not use a starter culture that has been stored at 4 ℃.
   Note: Do not grow starter culture directly from glycerol stock.

2. Harvest the bacterial by centrifugation at 5,000 x g for 10 minutes at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.

3. Add **2.5 mL Buffer A1** (Add RNase A to **Buffer A1** before use) and completely resuspend bacterial pellet by vortexing or pipetting **(Complete resuspension is critical for optimal yields).** Then add **125 µL Buffer ER** into the suspended bacterial culture. Mix well by inverting 5-10 times.

4. Add **2.25 mL Buffer B1,** mix gently but thoroughly by inverting 5 times and incubate for 5 minutes to obtain a slightly clear lysate to obtain a slightly clear lysate. Then add **250 µL Buffer D1**, mix gently and incubating for another 5 minutes.

   Note: Do not incubate longer than 5 minutes. Over-incubating causes genomic DNA contamination and plasmid damage.
   Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 50 ℃ to dissolve precipitation before use.

5. Add **1 mL Buffer N3**., mix immediately by inverting 5 times and sharp hand shaking for 5 times.

   Note: It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mix is required to completely neutralize the solution.

6. Transfer the lysate to a high-speed centrifuge tube and centrifuge at 12,000 x g for 10 minutes at room temperature.

Note: Syringe filter (Supplied in PD1422 or purchase separately from Biomiga) could be used to filtrate the lysate if high-speed centrifuge is not available.

7. Carefully transfer the clear lysate to a clean 15 mL tube (avoid the interface precipitates) and add **1 volume** of **Buffer RET (**For example, **5 mL** of **Buffer RET to 5 mL** of **clear lysate)**, and **3 mL** of **100% ethonal**. Mix well by sharp hand shaking for 5times. The mixture of ethanol/lysate needs to be transfer to the DNA column immediately.

8. Immediately transfer **6 mL** the **solution** into a DNA column with a 15 mL collection tube. Centrifuge at 5,000 x g for 2 min at room temperature. Remove the column from the tube and discard the flow-through liquid. Reinsert the column to the collection tube. Repeat till all the lysate/ethonal mix has been passed through the column.

   Note: If the 15 mL collection tube doesn't match the rotor (for example, the lid of rotor cannot close), the column with the collection can be centrifuged at a benchtop centrifuge at 3,000 rpm for 5 min. Swing bucket rotor is preferred.

9. Add **5 mL 70% ethanol** into the column, centrifuge at 5,000 x g for 1 min. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step 9.

10. Centrifuge the column at 5,000 x g, **with the lid open,** for 10 min to remove the ethanol residues.

11. Carefully transfer the spin column into a clean 15 mL tube and add **1 mL Endofree Elution Buffer** to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at 5,000 x g for 5 minutes.

12. Reload the elute into the center of the column and incubate for 1 minute. Elute the DNA by centrifugation at 5,000 x g for 5 minutes.

    Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, *in vitro* translation, sequencing, transfection, and microinjection.
    Note: Two elutions give rise to maximum DNA yield.
    DNA concentration (μg/mL) = $OD_{260\ nm}$ x 50 x dilution factor.

Purification of Low-Copy-Number Plasmid and Cosmid

The yield of low copy number plasmid is normally around 0.1 – 1 µg /mL of overnight culture. For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

1. Culture volume: Use **2 x volumes** of the **high copy number** culture. Use up to 100 mL for midipreps.

2. Use **2 x volumes** of the **Buffer A1**, **Buffer B1**, **Buffer N3**, **Buffer RET,** and **100% ethanol**. Additional buffers can be purchased from Biomiga.

3. Use **same volume** of **wash buffer (70% ethanol)** and **Endofree Elution Buffer**.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low Yield	Poor Cell lysis.	• Resuspend pellet thoroughly by votexing and pipetting prior adding Buffer B1. • Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2N NaOH and 1%SDS).
Low Yield	Bacterial culture overgrown or not fresh.	Grow bacterial 12-16 hours. Spin down cultures and store the pellet at -20℃. if the culture is not purified the same day. Do not store culture at 4℃ over night.
Low Yield	Low copy-number plasmid.	Increase culture volume and increase the volume of Buffer A1, B1, N3, and 100% ethanol according to instructions on page 8.
No DNA	Plasmid lost in Host *E.coli*	Prepare fresh culture.
Genomic DNA contamination	Over-time incubation after adding Buffer B1.	Do not vortex or mix aggressively after adding Buffer B1. Do not incubate more than 5 minutes after adding Buffer B1.
RNA contamination	RNase A not added to Buffer A1.	Add RNase A to Buffer A1.
Plasmid DNA floats out of wells while running in agarose gel, DNA doesn't freeze or smell of ethanol	Ethanol traces not completely removed from column.	Make sure that no ethanol residual remaining in the silicon membrane before eluting the plasmid DNA. Re-centrifuge again if necessary.

Limited Use and Warranty

This product is warranted to perform as described in its labeling and in Biomiga's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Biomiga. Biomiga's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Biomiga, to replace the products, Biomiga shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us at (858) 603-3219 or visit our website at www.biomiga.com

***FOR RESEARCH USE ONLY.**